

Pharmacokinetic Bioequivalence, Safety, and Immunogenicity of DMB-3111, a Trastuzumab Biosimilar, and Trastuzumab in Healthy Japanese Adult Males: Results of a Randomized Trial

Jun Morita¹ · Masashi Tanaka¹ · Masahiro Nomoto² · Shunji Matsuki³ · Tomomi Tsuru⁴ · Kyoko Matsuguma³ · Masanari Shiramoto³

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Abstract

Background DMB-3111 is a biosimilar trastuzumab drug being jointly developed by Meiji Seika Pharma (Japan) and Dong-A Socio Holdings (Korea). We investigated the bioequivalence of DMB-3111 relative to trastuzumab.

Objectives The aim of this study was to investigate the bioequivalence between DMB-3111 and trastuzumab and the pharmacokinetic, safety, and immunogenicity of both drugs in healthy Japanese adult males.

Methods Seventy healthy Japanese adult males were randomized 1:1 to receive either DMB-3111 or trastuzumab as a single intravenous infusion (6 mg/kg) over 90 min. Bioequivalence was assessed in terms of the pharmacokinetic parameters of both drugs. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Immunogenicity was tested using anti-drug antibody (ADA) assays.

Results The 90 % confidence intervals of the treatment differences (DMB-3111 versus trastuzumab) in the mean log-transformed maximum concentration, the area under the concentration–time curves (from 0 min to the last measured value or from 0 min to infinity), mean residence time, and the terminal half-life were within the accepted

range for bioequivalence [$\log(0.80)$ to $\log(1.25)$]. The frequencies of AEs and adverse drug reactions were similar with both drugs. No ADA reactivity to DMB-3111 or trastuzumab was observed in any subject.

Conclusions DMB-3111, a trastuzumab biosimilar, was bioequivalent to trastuzumab in terms of its pharmacokinetics and showed similar safety after a single intravenous infusion at 6 mg/kg over 90 min in healthy Japanese adult males. DMB-3111 is likely to show similar efficacy and safety profiles to trastuzumab in cancer patients (ClinicalTrials.gov #NCT02100917).

Key Points

DMB-3111, a trastuzumab biosimilar, and trastuzumab were founded to be pharmacokinetically bioequivalent.

The safety profile of DMB-3111 was similar to that of trastuzumab.

All obtained serum samples were negative for anti-drug antibody reactivity to either DMB-3111 or trastuzumab.

✉ Jun Morita
jun.morita@meiji.com

¹ Clinical Research Planning Department, Meiji Seika Pharma Co., Ltd., 2-4-16, Kyobashi, Chuo-ku, Tokyo 104-8002, Japan

² Pharmaceutical Research Center, Meiji Seika Pharma Co., Ltd., Yokohama, Japan

³ Sugioka Memorial Hospital, Souseikai, Fukuoka, Japan

⁴ PS Clinic, Souseikai, Fukuoka, Japan

1 Introduction

Trastuzumab is a humanized recombinant antibody that specifically targets human epidermal growth factor receptor 2 (HER2), which is often overexpressed in tumor cells [1–3]. Trastuzumab is believed to target HER2-

overexpressing tumor cells via two mechanisms. First, trastuzumab specifically binds to the extracellular domain of the HER2 receptor and induces antibody-dependent cell-mediated cytotoxicity (ADCC), which is mediated by natural killer cells and monocytes. Second, trastuzumab directly inhibits tumor cell growth by downregulating HER2 expression and inhibiting the HER2 signaling pathway involved in cell proliferation [1–3]. Trastuzumab is an effective treatment for patients with HER2-overexpressing metastatic breast cancer or curatively unresectable advanced/recurrent gastric cancer. It is also well established as adjuvant and neoadjuvant therapy for HER2-overexpressing breast cancer [2, 3].

DMB-3111 is a biosimilar drug to trastuzumab being co-developed by Meiji Seika Pharma Co., Ltd. (Japan) and Dong-A Socio Holdings Co. Ltd. (Korea). Like trastuzumab, DMB-3111 is produced using Chinese hamster ovary cells and is similar to trastuzumab in terms of their primary and secondary structures; sugar chain composition; molecular weight; electrophoretic and liquid chromatographic patterns; spectroscopic properties; osmotic pressure; water content; insoluble particulate matter; cell growth inhibitory activity; ADCC against BT-474 human breast carcinoma cells; and binding affinities for HER2, FcγRI, FcγRII, FcγRIII, FcRn, and C1q.

A biosimilar is a biologic product produced by an unrelated manufacturer/distributor with proven similarity to an approved biologic drug in terms of the quality, safety, and efficacy of both drugs [4]. Biosimilar products are approved on the basis that they show high similarity to the reference biological product. Only minor differences in clinically inactive components are allowed. Global regulatory registration requirements for a biosimilar drugs include demonstration of the safety and bioequivalence of the biosimilar drug to the original drug in clinical trials [4]. Recently, several biosimilar drugs have been assessed in terms of their bioequivalence to trastuzumab in healthy adults [5, 6]. Biosimilar products may show greater market availability owing to their lower cost.

The aim of this study was to investigate the bioequivalence between DMB-3111 and trastuzumab in terms of the pharmacokinetic (PK) properties, as well as the safety and immunogenicity of both drugs in healthy Japanese adult males.

2 Methods

This study was conducted at a single center between January 2014 and December 2014. The objective was to verify the PK bioequivalence of DMB-3111 and reference trastuzumab (Herceptin® for injection 150; 150 mg/vial;

Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and to confirm drug safety in healthy Japanese male adults.

2.1 Subjects

Healthy Japanese males aged 20–39 years with a body mass index of 17.6–26.4 kg/m² were eligible for the study. Before enrollment, the eligibility of each subject was checked at a screening visit at which physical examination, blood pressure, electrocardiography, echocardiography, and routine laboratory tests were performed. Subjects with a left ventricular ejection fraction of <60 %, as measured by echocardiography, history of hypersensitivity to components of trastuzumab or any other drug, and the use of any investigational drugs within 2 weeks before administration of DMB-3111 or trastuzumab were excluded.

The target number of subjects was set to allow us to verify the bioequivalence of both products using the pre-determined acceptance range for the PK parameters [i.e., the 90 % confidence interval (CI) for the log-transformed values being within the range of 0.8–1.25]. If the ratio between the PK parameters [maximum concentration (C_{max}) and area under the concentration–time curve from time 0 to the last measurable value (AUC_{0-t})] of DMB-3111 and trastuzumab is 1, and the coefficient of variation (CV) is 25 %, 28 subjects were needed for each group to provide a power of 90 % (i.e., the 90 % CI for the difference of the log-transformed values being within the range of 0.8–1.25). Based on an expected withdrawal rate of 20 %, we planned to enrol 35 subjects.

2.2 Study Design and Treatments

This was a randomized, double-blind, parallel-group study with a total post-dose follow-up time of 71 days. Subjects were randomized in a 1:1 ratio to either DMB-3111 or trastuzumab using a random numbers list by the study drug allocation manager, who was unblinded. Because DMB-3111 and trastuzumab look different, the drug allocation manager concealed the identities of both products at the time each dose was prepared to ensure both preparations had the same appearance and were indistinguishable. The drug allocation manager created a drug allocation table and an emergency key, placed them in a container, and sealed the container as soon as the investigational products were allocated. The container was maintained in a secure location until the time set for unsealing. The drug allocation manager prepared each drug and then handed it to the investigators for administration. Blinding was maintained by the drug allocation manager, clinical research pharmacist, and contract laboratories who did not disclose any data that may lead to subject identification until unblinding of the study. The drug allocation manager was not involved in

any recording of results or observations. On day 1, subjects were given a single intravenous infusion of 6 mg/kg of DMB-3111 (150 mg/vial) or trastuzumab over 90 min. We dissolved DMB-3111 or trastuzumab (6 mg/kg) in 280 ml of physiological saline for administration. The subjects in both groups received oral premedication with D-chlorpheniramine maleate 2 mg (MSD K.K., Tokyo, Japan) 30 min before the start of infusion. The administration procedures were identical for both study drugs.

The subjects were required to stay at the trial site and were supervised by the investigators from day 1 (1 day before study drug administration) to day 3 (72 h after study drug administration). The subjects were instructed to avoid consuming beverages containing caffeine or alcohol, grapefruits, and grapefruit juice from 3 days before admission. While at the trial site, the subjects were prohibited from smoking and performing strenuous exercise. The subjects were also prohibited from taking non-study drugs, excluding the pre-specified premedication and any drugs needed for the treatment of an adverse event (AE).

2.3 Clinical and Laboratory Assessments

The demographic characteristics of each subject were recorded at enrollment. During the 71-day study period, the subjects were questioned about any symptoms and underwent physical examinations, assessment of vital signs (supine blood pressure, pulse rate, and axillary temperature), echocardiography, 12-lead electrocardiography, and laboratory tests [hematology, blood chemistry, urinalysis, and serum human epidermal growth factor receptor 2 (HER2) protein]. These tests were generally performed at the following times: screening, on day 1, before and after study drug administration on day 1, on days 2–4, 6, 9, 16, 23, 44, and 71. To confirm the level of HER2 protein, serum was collected before study drug administration on day 1 and on days 16 and 71.

2.4 Measurement of Serum Drug Concentrations

Blood samples were collected pre dose, at 0.5, 1, 1.5, 3, 6, 8, 12, 24, 36, 48, and 72 h post dose, and at 6, 9, 16, 23, 44, and 71 days post dose. Blood samples (3 ml) were collected from the brachial vein, placed at room temperature for 30 min, and then centrifuged at $2000 \times g$ for 10 min. The resulting sera were stored at -50°C until assayed. The serum levels of DMB-3111 and trastuzumab were determined using validated enzyme-linked chemiluminescent assays at a contract laboratory (Covance Laboratories Ltd., Harrogate, North Yorkshire, UK). The quantification limits of both assays were 40 ng/ml. The inter-run and intra-run precision values of DMB-3111 ranged from 5.8 to 12.6 % and from 3.2 to 5.6 %, respectively, and those of

trastuzumab ranged from 10.7 to 16.0 % and from 5.0 to 12.7 %, respectively. These values were within the criteria shown in the guidelines for the ligand-binding assay [7, 8], suggesting that concentrations of DMB-3111 and trastuzumab measured by this analysis method were reliable.

2.5 Pharmacokinetic (PK) Analyses

The serum drug concentration versus time data for both drugs were used to calculate the following parameters in each subject: C_{\max} , AUC_{0-t} , AUC from 0 to infinity ($AUC_{0-\infty}$), mean residence time from time 0 to the last measurable value (MRT_{0-t}), and the terminal half-life ($t_{1/2}$). C_{\max} was recorded as the peak value observed in each subject. AUC_{0-t} was estimated using the trapezoidal rule. $t_{1/2}$ was determined by fitting a linear regression to the drug concentration–time curve. PK analyses were performed using PhoenixTM WinNonlin[®] software, version 6.1 (Certara, L.P., Princeton, NJ, USA). The PK parameters are expressed as the mean (standard deviation) or mean (range). The bioequivalence of DMB-3111 (test) relative to trastuzumab (reference) was accepted if the 90 % CI of difference in the mean log-transformed value (test reference) was within the range from log (0.80) to log (1.25) that we established based on *Guidelines for Bioequivalence Studies of Generic Products* [9].

2.6 Safety Assessments

Potential AEs that occurred any time after the start of study drug infusion until 71 days post dose were recorded. AEs classified as possibly or definitely related to the study drug were listed as adverse drug reactions (ADRs). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

2.7 Immunogenicity Testing

Immunogenicity was assessed using serum samples obtained at 16 and 71 days post dose. Electrochemiluminescence (ECL) assays were conducted in a two-stage approach using anti-drug antibodies (ADAs) that react to DMB-3111 or trastuzumab. If the ECL signal was above the threshold value in a screening assay, a confirmatory assay was performed to determine whether the response was positive or negative. Using a risk-based approach, it is appropriate to detect 5 % of false-positive results, rather than any false-negative results in the screening assay. The threshold (cut-point) was set using a parametric approach using the mean assay response (of ≥ 50 individuals) plus 1.645 standard deviations (1.645 is the 95th percentile of the normal distribution). In the confirmation assay, a confirmation cut-point was set using human sera with or

without spiking the sample with a low concentration of ADAs to determine the percent inhibition of the response required to indicate a positive response specific to ADAs.

3 Results

3.1 Subject Disposition

Of 169 volunteers who were screened, 109 eligible subjects were selected for the study. Four subjects withdrew consent and 105 subjects were admitted to the study site. Of these, 70 were to be treated with the study drug, three were included as reserve subjects, one did not satisfy the eligibility criteria, and 31 were admitted to the study but were not treated or reserved. A total of 70 subjects were randomized to either DMB-3111 ($n = 35$) or trastuzumab ($n = 35$), and all of these subjects completed the study. As shown in Table 1, both groups were well matched in terms of their baseline characteristics. All 70 subjects were included in the bioequivalence, safety, and immunogenicity analyses. There were no changes in the interim analyses or stopping guidelines after starting the study.

3.2 PK of DMB-3111 and Trastuzumab

Figure 1 shows the serum DMB-3111 and trastuzumab concentrations versus time after the intravenous dose. The PK parameters for both drugs are summarized in Table 2. The serum concentration versus time profiles of DMB-3111 and trastuzumab were very similar. The serum DMB-3111 concentration peaked at 1.5–6.0 h post dose and subsequently decreased over time, while the serum trastuzumab concentration peaked at 1.5–3.0 h post dose. The mean (range) values for C_{\max} [138.81 (104.12–185.51) vs. 139.93 (106.28–210.97) $\mu\text{g/ml}$], AUC_{0-t} [1369.3 (903.5–1900.6) vs. 1362.1 (1118.6–1750.6) $\mu\text{g}\cdot\text{day/ml}$], $AUC_{0-\infty}$ [1371.0 (901.0–1908.8) vs. 1363.7 (1119.0–1755.7) $\mu\text{g}\cdot\text{day/ml}$], MRT_{0-t} [297.8 (224.7–364.1) vs. 301.7 (235.1–352.6) h] and $t_{1/2}$ [7.07 (4.30–9.57) vs. 6.98 (5.54–9.39) days] were similar between DMB-3111 and trastuzumab, respectively.

Table 2 shows the 90 % CIs for difference in the log-transformed mean values of the PK parameters (DMB-3111—trastuzumab). The 90 % CIs for C_{\max} [$\log(0.9384)$ to $\log(1.0554)$], AUC_{0-t} [$\log(0.9431)$ to $\log(1.0624)$], $AUC_{0-\infty}$ [$\log(0.9429)$ to $\log(1.0627)$], MRT_{0-t} [$\log(0.9453)$ to $\log(1.0263)$], and $t_{1/2}$ [$\log(0.9450)$ to $\log(1.0777)$] were within the pre-specified range of log (0.80) to log (1.25) for accepting bioequivalence.

3.3 Safety and Immunogenicity

In both groups, 31 of 35 (88.6 %) subjects experienced an ADR, with a total of 115 and 108 AEs in the DMB-3111 and trastuzumab groups, respectively. Most of the AEs were of grade 1 or 2 severity; there were no grade 4 or 5 events. Grade 3 AEs occurred in two (5.7 %) subjects in both groups. The grade 3 AEs in the DMB-3111 group were pyrexia and increased serum amylase in one subject (2.9 %) each. Grade 3 AEs in the trastuzumab group were increased serum amylase and increased blood creatine phosphokinase in one subject (2.9 %) each. All grade 3 AEs, except for increased blood creatinine phosphokinase in the trastuzumab group, were classified as ADRs. No AEs were serious or led to study withdrawal.

Table 3 shows the proportions of subjects with ADRs in each group. The most frequent ADRs [DMB-3111 vs. trastuzumab; n (%) of subjects] were increased serum C-reactive protein (CRP) [25 (71.4) vs. 21 (60.0)], pyrexia [15 (42.9) vs. 14 (40.0)], and increased blood brain natriuretic peptide (BNP) [14 (40.0) vs. 13 (37.1)]. All episodes of increased CRP and BNP were classified as grade 1 in both groups. Decreased lymphocyte count [18 (51.4) vs. 19 (54.3)], increased WBC count [3 (8.6) vs. 3 (8.6)], and increased neutrophil percentage [21 (60.0) vs. 22 (62.9)] were also reported in similar proportions of subjects in both groups. All episodes of decreased lymphocyte count were classified as grade 1 or 2, while all episodes of increased WBC count and neutrophil percentage were classified as grade 1. Figures 2 and 3 show the mean values of clinical and laboratory parameters over time. The mean peak concentration (minimum–maximum) of the serum CRP concentrations of DMB-3111 and trastuzumab was 1.441

Table 1 Characteristics of healthy Japanese adult males receiving a single intravenous infusion of 6 mg/kg over 90 min of DMB-3111 or trastuzumab

Group	Age (years)	Height (cm)	Body weight (kg)	BMI (kg/m^2)
DMB-3111 ($n = 35$)	22.9 \pm 3.2	171.83 \pm 5.72	64.42 \pm 6.50	21.81 \pm 1.80
Trastuzumab ($n = 35$)	22.7 \pm 3.3	170.54 \pm 4.60	61.25 \pm 5.07	21.07 \pm 1.73

Values are presented as mean \pm standard deviation

BMI body mass index

Fig. 1 Serum drug concentration versus time profiles after a single intravenous infusion of 6 mg/kg of DMB-3111 or trastuzumab over 90 min in Japanese healthy males. Values are presented as the mean \pm standard deviation; $n = 35$ subjects per group

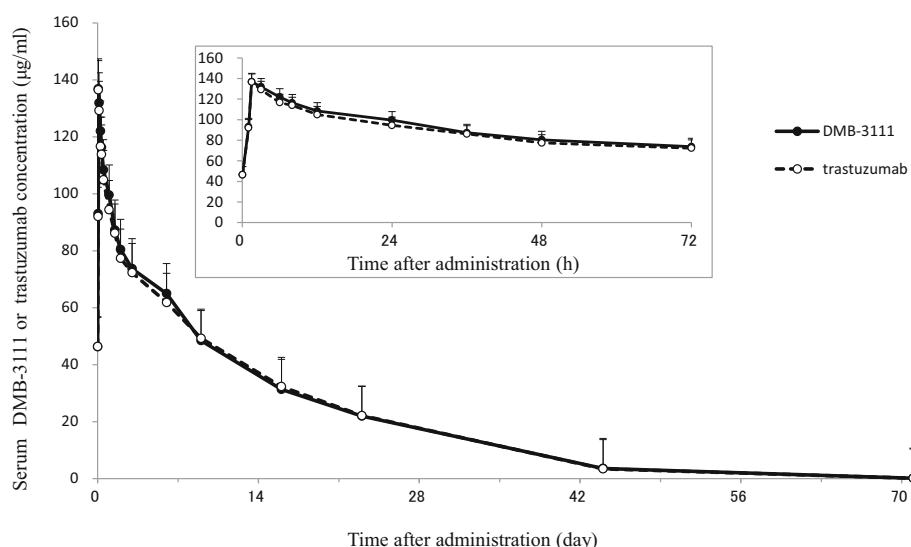


Table 2 Pharmacokinetic parameters and mean differences of DMB-3111 or trastuzumab after a single intravenous infusion of 6 mg/kg over 90 min in Japanese healthy adult males

Parameter	DMB-3111 ($n = 35$)	Trastuzumab ($n = 35$)	Difference (DMB-3111—trastuzumab)	90 % confidence interval
C_{\max} (µg/ml)	138.81 ± 19.22	139.93 ± 22.95	0.9952	0.9384–1.0554
AUC_{0-t} (µg·day/ml)	1369.3 ± 221.7	1362.1 ± 187.0	1.0010	0.9431–1.0624
$AUC_{0-\infty}$ (µg·day/ml)	1371.0 ± 223.4	1363.7 ± 187.8	1.0010	0.9429–1.0627
MRT_{0-t} (h)	297.8 ± 33.0	301.7 ± 27.0	0.9850	0.9453–1.0263
$t_{1/2}$ (days)	7.07 ± 1.23	6.98 ± 1.08	1.0092	0.9450–1.0777

Data are presented as mean \pm standard deviation unless otherwise indicated

AUC_{0-t} area under the concentration–time curve from 0 min to the last measured value, $AUC_{0-\infty}$ area under the concentration–time curve from 0 min to infinity, C_{\max} maximum concentration, MRT_{0-t} mean residence time from 0 min to the last measured value, $t_{1/2}$ terminal half-life

(0.01–6.44) mg/dl and 1.439 (0.01–5.40) mg/dl, respectively. Abnormal changes in echocardiography were not observed in either group.

All of the serum samples obtained at 16 and 71 days post dose were negative for ADA reactivity to either DMB-3111 or trastuzumab.

4 Discussion

We investigated the bioequivalence of DMB-3111, a biosimilar trastuzumab, to reference trastuzumab and evaluated the safety and immunogenicity of both drugs in healthy Japanese adult males. We found that both drugs were bioequivalent, had similar safety profiles, and did not induce the production of ADA reactive to DMB-3111 or trastuzumab in any subject.

DMB-3111 and trastuzumab had similar serum drug concentration versus time profiles. The test-to-reference differences in their PK parameters were from 0.9850 to

1.0092 (C_{\max} 0.9952; AUC_{0-t} 1.0010; $AUC_{0-\infty}$ 1.0010; MRT_{0-t} 0.9850; and $t_{1/2}$ 1.0092), and the upper and lower bounds of the 90 % CIs were around 1.0000 (Table 2). The smallest number of subjects needed to demonstrate the bioequivalence of the two products was calculated to be five, irrespective of whether bioequivalence was determined for C_{\max} or AUC_{0-t} . These results demonstrate that the PK profiles of DMB-3111 and trastuzumab are similar.

The PK properties of DMB-3111 and trastuzumab obtained in this study were similar to those reported for other biosimilar trastuzumab drugs and trastuzumab in healthy adults [5, 6, 10]. However, the previous bioequivalence studies of other biosimilar trastuzumab drugs reported wider CIs for the geometric mean test-to-reference ratios of the PK parameters (0.894–0.977 for C_{\max} and 0.896–1.001 for $AUC_{0-\infty}$), although these were within the accepted range for bioequivalence acceptance [5, 6]. The test-to-reference difference between DMB-3111 and trastuzumab in the present study was 0.9952 for C_{\max} and 1.0010 for $AUC_{0-\infty}$. These results suggest that the PK

Table 3 Incidence of adverse drug reactions after a single intravenous infusion of 6 mg/kg of DMB-3111 or trastuzumab over 90 min in Japanese healthy adult males

System organ class and preferred term	DMB-3111 (n = 35)			Trastuzumab (n = 35)			Total (n = 70)		
	Events (n)	Subjects (n)	Incidence (%)	Events (n)	Subjects (n)	Incidence (%)	Events (n)	Subjects (n)	Incidence (%)
Overall	115	31	88.6	108	31	88.6	223	62	88.6
Eye disorders	4	4	11.4	2	2	5.7	6	6	8.6
Ocular hyperemia	4	4	11.4	2	2	5.7	6	6	8.6
General disorders and administration site conditions	15	15	42.9	14	14	40.0	29	29	41.4
Pyrexia	15	15	42.9	14	14	40.0	29	29	41.4
Infections and infestations	0	0	0.0	1	1	2.9	1	1	1.4
Oral herpes	0	0	0.0	1	1	2.9	1	1	1.4
Investigations	86	31	88.6	84	31	88.6	170	62	88.6
Alanine aminotransferase increased	2	2	5.7	1	1	2.9	3	3	4.3
Amylase increased	1	1	2.9	1	1	2.9	2	2	2.9
Aspartate aminotransferase increased	1	1	2.9	1	1	2.9	2	2	2.9
C-reactive protein increased	25	25	71.4	21	21	60.0	46	46	65.7
Blood urine present	0	0	0.0	1	1	2.9	1	1	1.4
Lymphocyte count decreased	18	18	51.4	19	19	54.3	37	37	52.9
White blood cell count increased	3	3	8.6	3	3	8.6	6	6	8.6
Neutrophil percentage increased	21	21	60.0	22	22	62.9	43	43	61.4
Monocyte percentage decreased	1	1	2.9	2	2	5.7	3	3	4.3
Brain natriuretic peptide increased	14	14	40.0	13	13	37.1	27	27	38.6
Nervous system disorders	5	5	14.3	5	5	14.3	10	10	14.3
Headache	5	5	14.3	5	5	14.3	10	10	14.3
Respiratory, thoracic and mediastinal disorders	1	1	2.9	0	0	0.0	1	1	1.4
Epistaxis	1	1	2.9	0	0	0.0	1	1	1.4
Skin and subcutaneous tissue disorders	4	4	11.4	2	2	5.7	6	6	8.6
Acne	3	3	8.6	1	1	2.9	4	4	5.7
Eczema	1	1	2.9	0	0	0.0	1	1	1.4
Erythema	0	0	0.0	1	1	2.9	1	1	1.4

Adverse events were coded using the MedDRA/J version 17.0 and CTCAE v4.0

properties of DMB-3111 are closer to those of trastuzumab than are those of the other biosimilar trastuzumab drugs.

We evaluated the safety and immunogenicity of both drugs in this study, but we did not perform statistical analysis. We investigated the frequency of incidents and the tendency of intensity of those.

DMB-3111 and trastuzumab were well tolerated in healthy Japanese adult males, and did not cause any serious or grade 4/5 AEs or any AEs that required study discontinuation. Grade 3 ADRs were infrequent, occurring in two subjects in the DMB-3111 group (pyrexia and increased serum amylase in one subject each) and in one subject in the trastuzumab group (increased serum amylase). The types and frequencies of grade 1/2 ADRs were also similar in both groups. There were no differences in the profiles of these and other AEs or ADRs, including the frequency,

total number of episodes, grade, timing in relation to dosing, treatments provided, outcome, and time to recovery/resolution between the two treatment groups. The AE profiles of both drugs were also consistent with those of previous reports of trastuzumab and other biosimilar trastuzumab drugs [5, 6].

Among the most notable AEs associated with trastuzumab were infusion reactions, which occurred in about 40 % of trastuzumab-treated patients [2, 3]. In the present study, the ADR pyrexia, a symptom of infusion reaction, occurred in about 40 % of subjects in both groups. All of the episodes were transient and resolved within 1 day. The serum CRP concentrations increased after infusion and reached peak values on day 2. The mean peak concentration (minimum–maximum) in the DMB-3111 group was similar to that in the trastuzumab group [1.441 (0.01–6.44) vs.

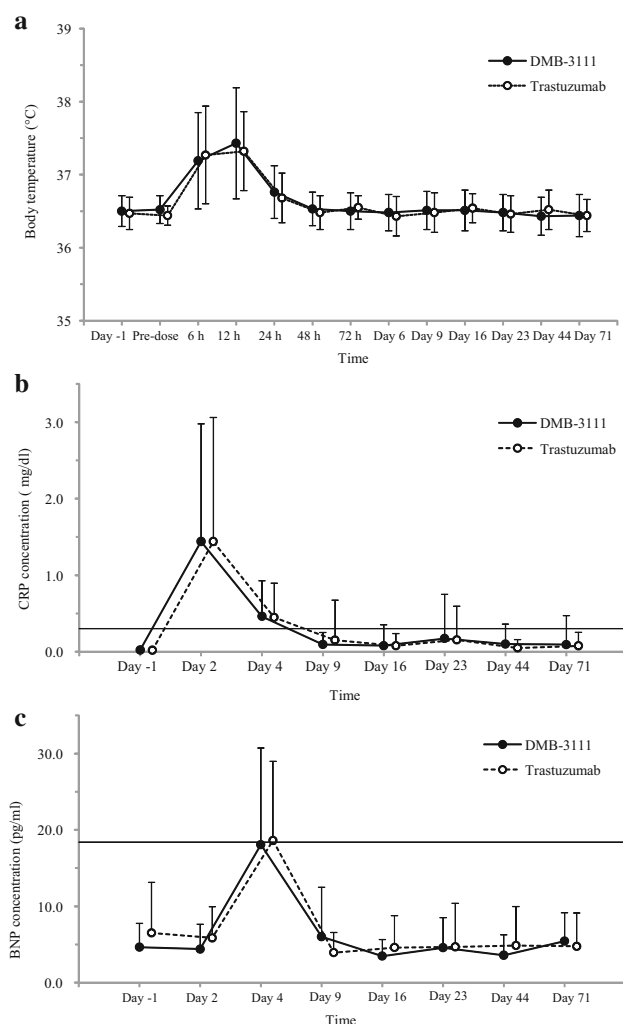


Fig. 2 Body temperature (a), C-reactive protein (CRP) concentrations (b), and brain natriuretic peptide (BNP) concentrations (c) after a single intravenous infusion of 6 mg/kg of DMB-3111 or trastuzumab over 90 min in Japanese healthy males ($n = 35$ in each group). Values are presented as the mean \pm standard deviation in (a) or mean + standard deviation in (b) and (c). Reference range: ≤ 0.30 mg/dL in (b) and ≤ 18.4 pg/ml in (c)

1.439 (0.01–5.40) mg/dl]. The serum CRP concentration declined to below the detectable limit during the follow-up period in all of the subjects.

Headache, another symptom of infusion reactions, occurred in five subjects (14.3 %) in both groups. All episodes were of grade 1 severity. Although one subject treated with trastuzumab experienced headache that lasted until day 2, the events resolved within 1 day in the remaining nine subjects. The frequency and timing of infusion reactions after administration of DMB-3111 or trastuzumab in this study were similar to those reported in cancer patients (mainly females) treated with trastuzumab [3], indicating there are no differences between cancer patients (mainly females) and healthy subjects regarding these events.

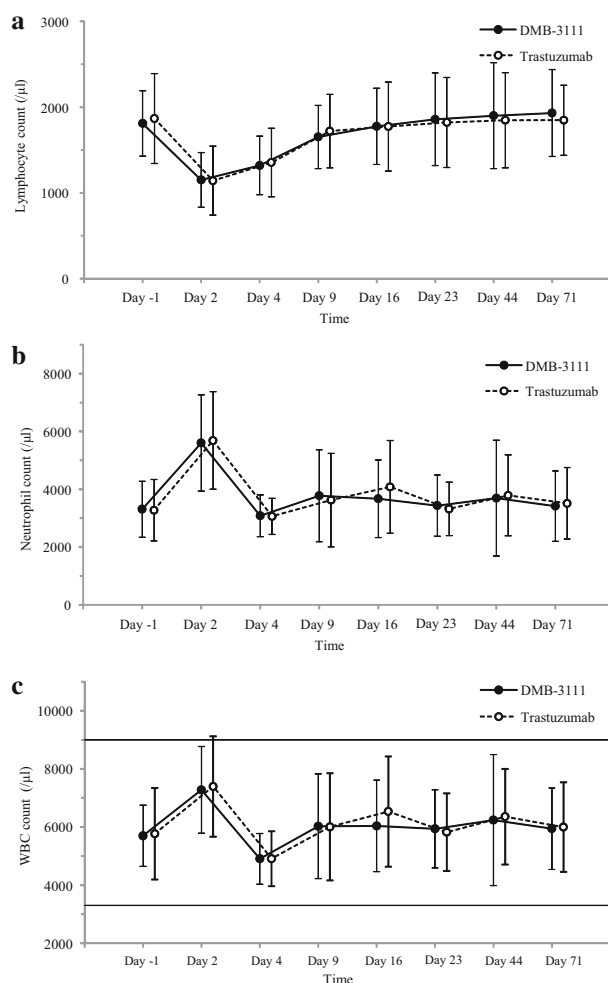


Fig. 3 Lymphocyte count (a), neutrophil count (b), and white blood cell (WBC) count (c) after a single intravenous infusion of 6 mg/kg of DMB-3111 or trastuzumab over 90 min in Japanese healthy males ($n = 35$ in each group). Values are presented as the mean \pm standard deviation. Reference range: 3300–9000/ μ L in (c)

Trastuzumab has been associated with cardiotoxic events, such as heart failure [2, 3, 11]. To reduce this risk, we excluded subjects with a history of or current cardiac disease or left ventricular ejection fraction of <60 % from the study, and regularly performed echocardiography and measured blood BNP concentrations (a biomarker of heart failure) after administration of the study drugs. We observed no echocardiographic abnormalities during the follow-up period, but observed ADR increases in blood BNP concentrations in both groups. Before each drug administration, the mean blood BNP concentrations were <10 pg/ml. The mean blood BNP concentration reached a peak on day 4, and the peak concentration (minimum–maximum) in the DMB-3111 group was similar to that in the trastuzumab group [18.07 (2.0–64.8) vs. 18.62 (3.8–44.1) pg/ml] (Fig. 2). The blood BNP concentration decreased to below the detectable limit during the follow-

up period in all of the subjects. Because a blood BNP concentration of 100 pg/ml has been proposed as a threshold for screening for overt heart failure [12], single doses of DMB-3111 and trastuzumab are unlikely to cause organic changes in the heart. As the range of the serum CRP concentrations after administration of DMB-3111 or trastuzumab was very large, the mean, minimum, and maximum data were similar to those in subjects receiving trastuzumab.

The other ADRs reported in this study included decreased lymphocyte count on days 2 or 4, WBC count increased on day 2, and neutrophil percentage increased on day 2 in both groups. The lymphocyte count completely normalized during the follow-up period in all subjects receiving trastuzumab except for one subject in whom the lymphocyte count returned towards the normal value on the last day of the follow-up period. The WBC count and neutrophil percentage returned to normal values within 3 days in all subjects in both groups. It was thought that the CRP and BNP after administration of DMB-3111 showed the same tendency as those after administration of trastuzumab.

All serum samples obtained at 16 and 71 days after treatment with DMB-3111 or trastuzumab were negative for ADA reactivity to either drug. ADA reactivity was reported in 1/921 cancer patients treated with trastuzumab [2, 3] and in 1/35 healthy adult subjects treated with trastuzumab in a study of a trastuzumab biosimilar [5]. However, neither of the two trastuzumab biosimilar drugs used in prior studies elicited ADA reactivity [5, 6]. In our study, we observed no ADA reactivity to DMB-3111 or trastuzumab after a single dose of either drug. These results indicate that DMB-3111 has a similar immunogenicity profile to that of trastuzumab and other biosimilar drugs.

We investigated the similarity of DMB-3111, a trastuzumab biosimilar, and trastuzumab PKs and safety in males who are not likely to be affected by hormone cycles, etc., under limited and strict conditions. As a result, the study showed that DMB-3111 and trastuzumab were equivalent, and there was no inconsistency in safety between the current results and those obtained in the previous studies that were conducted mainly in female patients. Accordingly, we concluded that DMB-3111 administration would produce similar results as would trastuzumab administration.

5 Conclusions

This study showed that DMB-3111 was comparable to reference trastuzumab in terms of PK and safety profiles after a single intravenous infusion of 6 mg/kg over 90 min in healthy Japanese adult males. These results suggest that DMB-3111 may show efficacy and safety profiles similar

to trastuzumab in cancer patients. In this study, DMB-3111 was administered as a single intravenous infusion to healthy adult males. These promising results warrant further development of this trastuzumab biosimilar, and may offer the possibility of increasing the availability of highly effective treatments for patients with HER2-positive malignancies.

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Compliance with ethical standards

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Conflicts of interest J. Morita, M. Tanaka, and M. Nomoto are employees of Meiji Seika Pharma Co., Ltd. S.Matsuki, T. Tsuru, K. Matsunaga, and M. Shiramoto have no conflicts of interest to declare.

Ethical approval The study protocol and anticipated AEs were explained to the subjects using an informed consent document approved by the Institutional Review Board at Kyushu Clinical Pharmacology Research Clinic. All of the subjects gave written informed consent to participate in the study. The Institutional Review Board at Kyushu Clinical Pharmacology Research Clinic reviewed the ethical, scientific, medical, and pharmaceutical validity of the study and approved the study protocol. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice Guidelines, and the study protocol (ClinicalTrials.gov #NCT02100917). Eligible subjects were selected for the study and enrolled at Kyushu Clinical Pharmacology Research Clinic.

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References

1. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. *N Eng J Med*. 2007;357:39–51.
2. Herceptin® for Injection. Package Insert, 23rd edn. Tokyo: Chugai Pharmaceutical Co., Ltd.; 2013.
3. Herceptin® for Injection. Interview Form, 18th edn. Tokyo: Chugai Pharmaceutical Co., Ltd.; 2013.
4. Guidelines for approval of biosimilars. National Institute of Health Sciences, Tokyo, Japan. <http://www.nihs.go.jp/dbcb/TEXT/yakusyokuhatu-0304004.pdf>.
5. Yin D, Barker KB, Li R, Meng X, Reich SD, Ricart AD, Rudin D, Taylor CT, Zacharchuk CM, Hansson AG. A randomized phase 2 pharmacokinetic trial comparing the potential biosimilar PF-05280014 with trastuzumab in healthy volunteers (REFLECTIONS B327-01). *Br J Clin Pharmacol*. 2014;78:1281–90.
6. Wisman LA, De Cock EP, Reijers JA, Kamerling IM, Van Os SH, de Kam ML, Burggraaf J, Voortman G. A phase I dose-

- escalation and bioequivalence study of trastuzumab biosimilar in healthy male volunteers. *Clin Drug Investig.* 2014;34:887–94.
7. Guidance for Industry, Bioanalytical Method Validation Draft guidance. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM); 2013.
 8. Guideline on Bioanalytical Method Validation. Committee for Medicinal Products for Human Use (CHMP); 2011.
 9. Guidelines for Bioequivalence Studies of Generic Products. Pharmaceuticals and Medical Devices Agency, Tokyo, Japan. 2015. <http://www.pmda.go.jp/files/000157415.pdf>. Accessed 12 June 2015.
 10. Wynne C, Harvey V, Schwabe C, Waaka D, McIntyre C, Bittner B. Comparison of subcutaneous and intravenous administration of trastuzumab: a phase I/Ib trial in healthy male volunteers and patients with HER2-positive breast cancer. *J Clin Pharmacol.* 2013;53:192–201.
 11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001;344:783–92.
 12. Pichon MF, Cvitkovic F, Hacene K, Delaunay J, Lokiec F, Collignon MA, Pecking AP. Drug-induced cardiotoxicity studied by longitudinal B-type natriuretic peptide assays and radionuclide ventriculography. *In Vivo.* 2005;19:567–76.